

gene, wherein said hairpin RNA (i) is a substrate for cleavage by a RNaseIII enzyme to produce a double-stranded RNA product, (ii) does not produce a general sequence-independent killing of the mammalian cells, and (iii) reduces expression of said target gene in a manner dependent on the sequence of said complementary regions.

49. **(amended)** A method for attenuating expression of a target gene in mammalian cells, comprising introducing into the mammalian cells a single-stranded hairpin ribonucleic acid (RNA) comprising self complementary sequences of 19 to 100 nucleotides that form a duplex region, which self complementary sequences hybridize under intracellular conditions to a target gene, wherein said hairpin RNA (i) is cleaved in the mammalian cells to produce an RNA guide sequence that enters an Argonaut-containing complex, (ii) does not produce a general sequence-independent killing of the mammalian cells, and (iii) reduces expression of said target gene in a manner dependent on the sequence of said complementary regions.
50. **(amended)** The method of claim 48, 49 or 83, wherein the hairpin RNA is transfected into said mammalian cells.
51. **(amended)** The method of claim 48, 49 or 83, wherein the hairpin RNA is microinjected into said mammalian cells.
52. **(amended)** The method of claim 48, 49 or 83, wherein the hairpin RNA is a transcriptional product that is transcribed from an expression construct introduced into said mammalian cells, which expression construct comprises a coding sequence for transcribing said hairpin RNA, operably linked to one or more transcriptional regulatory sequences.

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56. **(amended)** The method of claim 53, wherein said promoter is selected from the group consisting of a T7 promoter, a T3 promoter, and an SP6 promoter.
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60. **(amended)** The method of claim 48, 49 or 83, wherein the mammalian cells are germ line cells.

61. **(amended)** The method of claim 48, 49 or 83, wherein the mammalian cells are stem cells.

62. **(amended)** The method of claim 48, 49 or 83, wherein the mammalian cells are somatic cells.

63. **(amended)** The method of claim 48, 49 or 83, wherein the mammalian cells are immortalized cells.

64. **(amended)** The method of claim 48, 49 or 83, wherein the mammalian cells are primate cells.

65. **(amended)** The method of claim 64, wherein the primate cells are human cells.

66. **(amended)** The method of claim 48, 49 or 83, wherein the mammalian cells are selected from the group consisting of adipocytes, fibroblasts, myocytes, cardiomyocytes, endothelium, neurons, glia, blood cells, megakaryocytes, lymphocytes, macrophages, neutrophils, eosinophils, basophils, mast cells, leukocytes, granulocytes, keratinocytes, chondrocytes, osteoblasts, osteoclasts, hepatocytes, and cells of the endocrine or exocrine glands.

81. **(amended)** The method of claim 48, 49 or 83, wherein the self complementary sequences are 20-50 nucleotides in length.

82. **(amended)** The method of claim 48, 49 or 83, wherein the self complementary sequences are 29 nucleotides in length.

83. **(amended)** A method for attenuating expression of one or more target genes in mammalian cells, comprising introducing into the mammalian cells a variegated library of single-stranded hairpin ribonucleic acid (RNA) species, each hairpin RNA species comprising self complementary sequences of 19 to 100 nucleotides that form duplex regions and which hybridize under intracellular conditions to a target gene, wherein each of said hairpin RNA species (i) is a substrate for cleavage by a RNaseIII enzyme to produce a double-stranded RNA product, (ii) does not produce a general sequence-independent killing of the mammalian cells, and (iii) if complementary to a target sequence, reduces expression of said target gene in a manner dependent on the sequence of said complementary regions.

87. **(new)** The method of claim 83, including the further step of identifying hairpin RNA species of said variegated library which produce a detected phenotype in said mammalian cells.

88. **(new)** The method of claim 55, wherein said promoter is an RNA polymerase III promoter or an snRNA promoter.

89. **(new)** The method of claim 88, wherein said promoter is an U6 promoter.